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Increased Risk of Atrial Fibrillation with Attenuated Activity of P21-Activated Kinase
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The mechanisms underlying atrial fibrillation (AF) are not fully understood. In a canine model of chronic atrial fibrillation (AF) we determined that the p21-activated kinase1 (Pak1) was down-regulated compared to animals in sinus rhythm. The down-regulation was mimicked in an in-vitro model of AF (atrial myocytes + Angiotensin II (AngII: 1μM, 24 h) leading us to hypothesize that attenuated Pak1 activity enhances atrial arrhythmic activity. ECG recordings in WT and Pak1-KO mice performed under control conditions and during stimulation with AngII or Carbachol (CCh: 150 ng/g) revealed spontaneous atrial arrhythmic events in Pak1-KO mice. In field stimulated isolated atrial myocytes (AM) an increased number of delayed after-depolarizations was determined (DADs WT: 14%; Pak1-KO: 39% of cells). AMs with attenuated Pak1 activity (Pak1-KO or WT + IPA3 : 10μM) exhibited an exaggerated increase in AngII induced ROS production (DCF, ΔF/F0: WT(ctl) 1.33 ± 0.07, WT(IPA3) 1.77 ± 0.04 , Pak1-KO 1.77 ± 0.08) that was attenuated in the presence of the NOX2 inhibitor apocynin (100 μM). The exaggerated AngII induced increase in Ca transient amplitude (ΔF/F0: WT(ctl) 2.6 ± 0.2, WT(AngII) 3.4 ± 0.4, Pak1-KO(ctl) 2.7 ± 0.3, Pak1-KO(AngII) 4.1 ± 0.4; $p < 0.05$) was also suppressed by NOX2 inhibition despite the fact that the increase in Ca depended on its release from inositol 1,4,5-trisphosphate sensitive stores (2-APB: 2μM). Pak1 stimulation in WT cells by FTY720 (200 nM) attenuated the AngII induced ROS production as well as the increase in Ca transients.
In conclusion, attenuated Pak1 activity in atrial tissue increases the likelihood of AF. Pak1 stimulation attenuates NOX2 dependent ROS production and the subsequent facilitation of IP3R mediated Ca release.

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Pro-Arrhythmic Calcium Waves Induced by Phosphodiesterase Type 4 Inhibition upon Beta-Adrenergic Stimulation Involve Both PKA and CamkII
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β-adrenergic receptor (β-AR) stimulation increases cardiac function by increasing cAMP levels and activating protein kinase A (PKA). PKA enhances Ca^{2+}-induced Ca^{2+}-release by phosphorylating L-type Ca^{2+} channels, ryanodine receptors and phospholamban (PLB) which are also targets of the Ca^{2+}/Calmodulin Kinase II (CaMKII). Any dysregulation in the β-adrenergic pathway leads to cardiac arrhythmias. Multiple cyclic nucleotide phosphodiesterases (PDEs) regulate local concentrations of cAMP, among which the PDE4 family is overriding in rodent heart. We investigated the proarrhythmic effects of PDE4 inhibition and evaluated the relative contribution of PKA and CaMKII to this mechanism. Action potentials were recorded at a frequency of 1Hz in isolated adult rat ventricular myocytes using the patch-clamp technique. Delayed afterdepolarizations (DADs) observed upon application of the non selective β-AR agonist Isoproterenol (Iso 1nM) after cessation of electrical stimulation during 10s were potentiated by the PDE4 inhibitor Ro20-1724 (Ro 10μM). These DADs correlated with spontaneous calcium waves (SCWs), recorded in myocytes loaded with Fura-2AM (1μM) using an Ionoptix system. Ro potentiated the inotropic and lusitropic effects of Iso and furthered sarcoplasmic reticulum (SR) Ca^{2+} load leading to SR Ca^{2+} leak measured in a $0Na^{+}$,$0Ca^{2+}$ solution ± 1mM tetracaine. Upon PDE4 inhibition, PLB was phosphorylated not only by PKA but also by CaMKII demonstrating that both kinases were activated. PKA inhibition with H-89 (10μM) suppressed the SCWs and the inotropic and lusitropic effects of Iso ± Ro. CaMKII inhibition with KN-93 (10μM) diminished SCWs incidence by 72% without affecting the inotropic effects of Ro. Thus, upon β-AR stimulation, PDE4 inhibition exerts positive inotropic effects via PKA but induces SCWs and DADs via both PKA and CaMKII activation, suggesting the potential use of CaMKII inhibitors as an adjunct to PDE inhibition in cardiac diseases to limit arrhythmias.

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Serca Stimulation Increases Intra-Sr Ca^{2+}Threshold for Ca^{2+} Waves in Cadiomyocytes
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In cardiac muscle, stimulation of β-adrenergic receptors leads to activation of PKA via the second messenger cAMP. PKA-dependent phosphorylation of several targets subsequently amplifies the L-type Ca^{2+} current, relieves the SERCA inhibition by PLB and is proposed to increase the Ca^{2+} sensitivity of the RyRs (by phosphorylation at serine 2808). Together, these mechanisms account for positive inotropy, but can also be arrhythmogenic via facilitation of spontaneous Ca^{2+} waves. Unexpectedly, the level of Ca^{2+} inside the SR ($[Ca^{2+}]_{SR}$) needed to initiate such waves has been reported to increase upon β-adrenergic stimulation, an observation which cannot be easily reconciled with elevated Ca^{2+} sensitivity of the RyRs. We tested the hypothesis that this change of Ca^{2+} wave threshold could occur indirectly, subsequent to SERCA disinhibition. Cytosolic and intra-SR Ca^{2+} waves were simultaneously recorded with confocal line-scans in permeabilized mouse cardiomyocytes with rhod-2 and fluo-5-N, respectively. We analyzed changes of SR Ca^{2+} content and several Ca^{2+} wave parameters. Ochratoxin A (OTA) was used to specifically stimulate the SERCA and the findings were compared to those in the presence of cAMP, which besides SERCA stimulation also leads to RyR phosphorylation. While cAMP resulted in a modest increase of Ca^{2+} wave thresholds (F/F_0 9% ± 3.5%), OTA lead to a substantial increase (30 ± 5.1%). Both compounds resulted in an acceleration of SR refilling (OTA earlier than cAMP), confirming SERCA stimulation. While cAMP provoked in the occurrence of Ca^{2+} sparks and mini waves, possibly accounting for the less pronounced SR loading, this was not observed in OTA. Together these results suggest that SERCA stimulation alone can elevate the intra-SR threshold for the generation of Ca^{2+} waves, independently of RyR phosphorylation. Supported by SNF.

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Calcium Handling is Altered in the Actc E99K Transgenic Mouse Model of Hypertrophic Cardiomyopathy
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The ACTC (cardiac actin) E99K transgenic (TG) mouse reproduces many aspects of hypertrophic cardiomyopathy (HCM) seen in humans. The most striking characteristic is the high probability of sudden cardiac death (SCD) in young mice (28-45 days old) probably due to arrhythmias, but how this arises from a myofilament mutation remains unknown. Previous studies demonstrated that the primary effect of this mutation is increased myofilament Ca^{2+} sensitivity. We hypothesise that increased sensitivity will disturb intracellular Ca^{2+} homeostasis and trigger afterdepolarisations.
Ventricular myocytes, enzymatically dissociated from E99K TG and non-transgenic (NTG) mice aged 8-12 weeks old were externally stimulated. Myocytes from TG animals showed prolonged relaxation (R50=0.079 ± 0.003s vs 0.062 ± 0.003s, $P < 0.001$) with no differences in maximum sarcomere shortening. Loaded with Fura-2AM, TG cells had smaller Ca^{2+} transients (Ratio=0.417 ± 0.023 vs 0.519 ± 0.022, $P < 0.01$) and SR load than their NTG littermates (R=1.087 ± 0.071 vs 1.380 ± 0.0661, $P < 0.01$). We repeated these studies using cells isolated from mice aged 25-45 days old. Cells from TG mice had greater sarcomere shortening (0.097 ± 0.004μm vs 0.070 ± 0.003μm, $P < 0.001$) and faster relaxation than their NTG littermates (R50 = 0.064 ± 0.002s vs 0.072 ± 0.002s, $P < 0.05$). Ca^{2+} transient amplitude was greater in TG cells (R=0.641 ± 0.035 vs 0.389 ± 0.015, $P < 0.001$) however there were no differences in SR load. Calcium wave frequency after 5Hz stimulation was increased in TG cells (0.285 ± 0.018s^{-1} vs 0.175 ± 0.014s^{-1}, $P < 0.001$). In both age groups, cells from TG mice displayed reduced diastolic $[Ca^{2+}]$, slower efflux of Ca^{2+} via the Na^{+}/Ca^{2+} exchanger and no differences in SR Ca^{2+}-ATPase function.
These data show the cardiomyocytes from young TG mice are hypercontractile with increased Ca^{2+} transient amplitude which, coupled with increased propensity for spontaneous Ca^{2+} release, could lead to the formation of afterdepolarisations, predisposing the heart to potentially fatal arrhythmias.

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Multispot Multiphoton Ca^{2+} Imaging in Acute Myocardial Slices of CPVT Hearts
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Rationale: Alterations in cardiomyocyte (CM) Ca^{2+} handling play a role in initiating and sustaining inherited arrhythmias (e.g. Catecholaminergic Polymorphic Ventricular Tachycardia, CPVT). Most of the current data on Ca^{2+} handling properties have been obtained in isolated cells. Aims: We aimed to establish a multicellular cardiac model to investigate the complex Ca^{2+} dynamics in cardiomyocytes, taking advantage of multispot multiphoton microscopy. Methods and results: Acute thick (450 μm) ventricular slices were